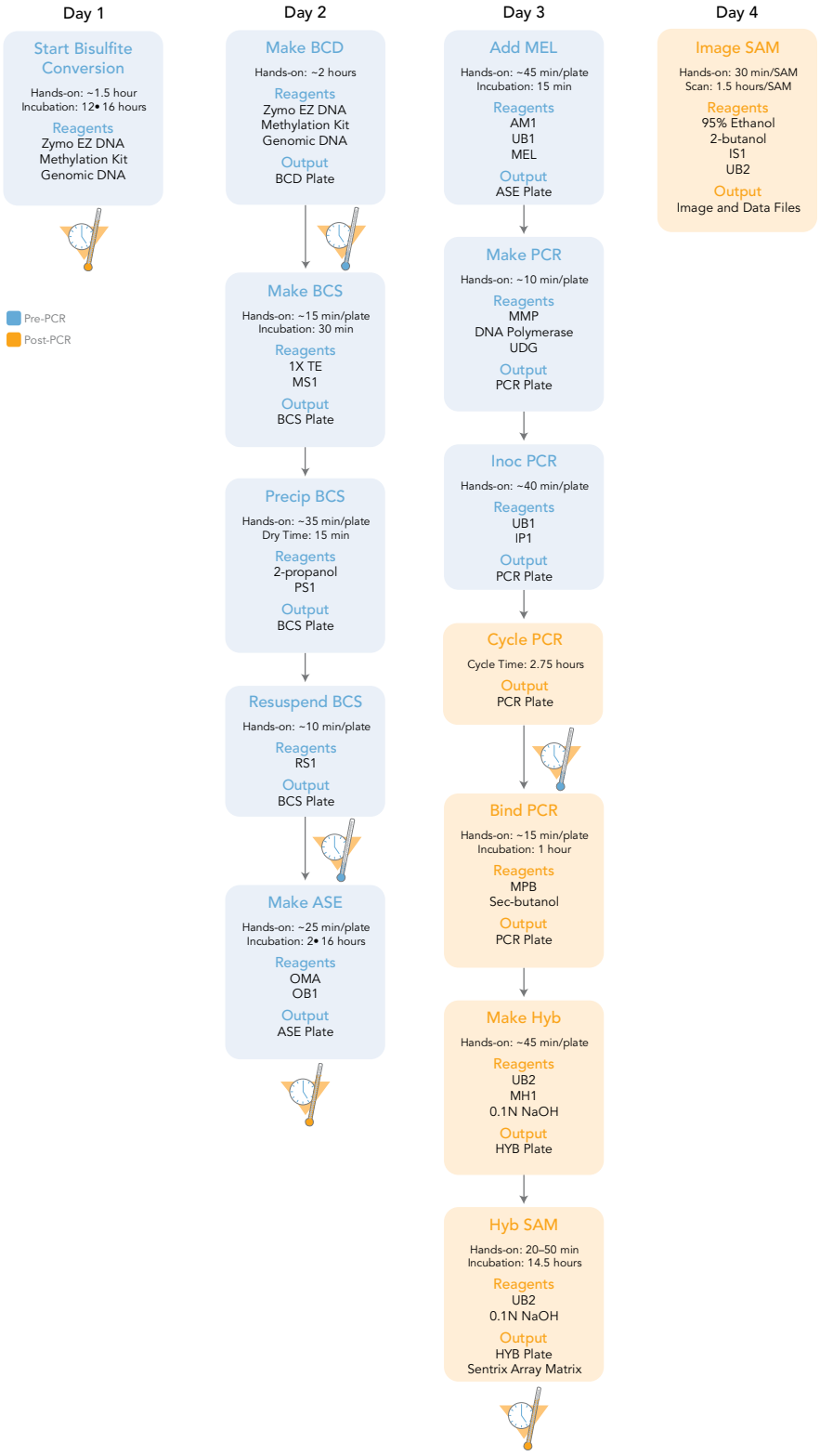
GoldenGate® Assay for Methylation, Single-Use, Manual

Experienced User Card



Part # 11233109 Rev. A

Make BCD

Hands-on time: 1.5 hours on Day 1
2 hours on Day 2

Incubation time: 12–16 hours

Bisulfite-convert the genomic DNA samples using the Zymo EZ DNA Methylation Kit. Transfer the bisulfite-converted samples to the BCD plate.

New Materials	Quantity
Zymo EZ DNA Methylation kit *(includes bisulfite-conversion reagent, dilution buffer, desulphonation buffer, elution buffer)*	1 kit per 2 plates
96-well 0.2 ml skirted microplate	1 to 3 plates
Genomic DNA	≥ 500 ng for each bisulfite conversion reaction

Preparation

- ☐ Prepare the conversion reagent according to the manufacturer's instructions. For best results, use it immediately.

  The conversion reagent is photosensitive, so you should minimize its exposure to light.

- ☐ Prepare the wash buffer according to the manufacturer's instructions.
- ☐ Apply a BCD barcode to each new plate.

Steps

Day 1

☐ **1.** Follow the instructions in the Zymo EZ DNA Methylation Kit to denature the genomic DNA and add conversion reagent.

Note: Denaturation is necessary for bisulfite conversion, since the conversion reagent only works on single-stranded DNA.

☐ **2.** Incubate in a light-protected area for 12–16 hours at 50°C.

Good stopping point

Day 2

- [ ] **3.** Follow the instructions in the Zymo EZ DNA Methylation Kit to do the following:
  - [ ] **a.** Wash off the conversion reagent.
  - [ ] **b.** Desulphonate the DNA in the column or plate. Incubate at room temperature (22°C) for 15 minutes.
  - [ ] **c.** Wash off the desulphonation buffer.
  - [ ] **d.** Add elution buffer.
- [ ] **4.** Centrifuge to elute.
- [ ] **5.** Transfer the bisulfite-converted DNA samples to the BCD plate.
- [ ] **6.** Heat-seal the plate and store at -20°C.

Do not store bisulfite-converted DNA for more than one month.

Good stopping point

Next step

Proceed to Make BCS.

Make BCS

Estimated processing time: 15 minutes

Incubation time: 30 minutes

Aliquot bisulfite-converted genomic DNA into a microtiter plate. Biotinylate converted DNA so that it will bind to the beads later on.

New Materials	Quantity
96-well 0.2 ml microtiter microplate	1 plate for each BCD plate
MS1 reagent	1 tube per BCS plate, -20°C

Preparation

- ☐ Preheat the heat block to 95°C. Allow 45 minutes.
- ☐ Turn on the heat sealer to preheat it.
- ☐ Thaw the MS1 reagent and the BCD plate to 22°C, if frozen.
- ☐ Apply a BCS barcode to a new plate. Use one plate for each BCD plate.

Steps

- ☐ **1.** Vortex the MS1 reagent.
- ☐ **2.** Add 5 µl MS1 to each well of the BCS plate.
- ☐ **3.** Add 5 µl bisulfite-converted DNA sample (for each 250 ng before conversion) to each well of the BCS plate.
- ☐ **4.** Heat seal the plate.
- ☐ **5.** Pulse centrifuge the plate to 250 xg.
- ☐ **6.** Vortex the plate at 2300 rpm (actual vortex speed) for 20 seconds.
- ☐ **7.** Pulse centrifuge the plate to 250 xg.
- ☐ **8.** Place the BCS plate in the 95°C heat block and close the heat block cover. Incubate for 30 minutes.

  Do not leave the plate in the heat block for more than 30 minutes.
- ☐ **9.** Pulse centrifuge the BCS plate to 250 xg.
- ☐ **10.** If you plan to proceed to Make ASE immediately after preparing the DNA, switch the heat block to 70°C now.

Next step

Proceed to Precip BCS.

Precip BCS

Estimated processing time: 30 minutes

Dry time: 15 minutes

Precipitate the DNA samples to remove any free biotin.

New Materials	Quantity
PS1 reagent	Bottle (1 ml), 22°C
2-propanol	Bottle (2 ml), 22°C

Steps

- ☐ **1.** Add 5 µl PS1 reagent to each well of the BCS plate.
- ☐ **2.** Seal the plate with adhesive film.
- ☐ **3.** Pulse centrifuge the plate to 250 xg.
- ☐ **4.** Vortex the plate at 2300 rpm for 20 seconds.
- ☐ **5.** Add 15 µl 2-propanol to each well of the BCS plate.
- ☐ **6.** Seal the plate with adhesive film.
- ☐ **7.** Vortex the plate at 1600 rpm for 20 seconds or until the wells are uniformly blue.
- ☐ **8.** Centrifuge the sealed plate to 3000 xg for 20 minutes.

  Perform the next step immediately to avoid dislodging the activated DNA pellets. If any delay occurs, recentrifuge to 3000 xg for 10 minutes before proceeding.
- ☐ **9.** Remove the plate seal.
- ☐ **10.** Decant the supernatant by quickly inverting each BCS plate and tapping it firmly onto an absorbent pad. Blot off excess fluid.
- ☐ **11.** Tap firmly several times over a period of 1 minute or until all wells are devoid of liquid.

  Do not allow supernatant to pour into other wells.
- ☐ **12.** Place the inverted BCS plate on a new absorbent pad. Centrifuge to 8 xg for 1 minute.

  Do not centrifuge to more than 8 xg, or the sample will be lost!
- ☐ **13.** Set the plate upright and allow to air-dry for 15 minutes.

Next step

Proceed to Resuspend BCS.

Resuspend BCS

Estimated processing time: 15 minutes

Dissolve biotinylated bisulfite-converted DNA pellets and resuspend them in solution.

New Materials	Quantity
RS1 reagent	Bottle, 22°C

Preparation

- ☐ Thaw the RS1 reagent to 22°C and invert several times to mix. Allow 1–3 hours to thaw.

Steps

- ☐ **1.** Add 10 µl RS1 to each well of the BCS plate.
- ☐ **2.** Seal the plate with adhesive film.
- ☐ **3.** Pulse centrifuge to 250 xg.
- ☐ **4.** Vortex at 2300 rpm for 1 minute or until the blue DNA pellets are completely resuspended.

Good stopping point

Next step

Do one of the following:

- Proceed to Make BCS ASE.
- Heat-seal the BCS plate and store at 4°C overnight.

Make BCS ASE

Estimated processing time: 20 minutes

Incubation time: 2–16 hours (if using heat block)
16 hours (if using hyb oven)

Bind allele-specific oligos (ASOs) and locus-specific oligos (LSOs) to the biotinylated bisulfite-converted genomic DNA. Bind the DNA to paramagnetic beads that are coated with streptavidin.

New Materials	Quantity
96-well 0.2 ml microtiter plate	1 plate for each BCS plate
OMA	1 tube per plate, -20°C
OB1	1 tube per plate, -20°C

Preparation

- ☐ Preheat the heat block to 70°C. Allow 45 minutes.
- ☐ Turn on the heat sealer to preheat it. Allow 15 minutes.
- ☐ Thaw the OMA and OB1 reagents to 22°C.
- ☐ Apply a ASE barcode to a new plate, one for each BCS plate.
- ☐ If you stored the BCS plate at 4°C, thaw it to 22°C.

Steps

- ☐ **1.** Vortex the OMA reagent.
- ☐ **2.** Pulse centrifuge the OMA reagent to 250 xg, and then pour it into a reagent reservoir.
- ☐ **3.** Add 10 µl OMA to each well of the new ASE plate.
- ☐ **4.** Vortex the OB1 reagent to resuspend the beads. Pour into a reservoir.
- ☐ **5.** Add 30 µl OB1 to each well of the ASE plate.
- ☐ **6.** Transfer 10 µl activated DNA from each well of the BCS plate to the corresponding well in the ASE plate. Discard the BCS plate.
- ☐ **7.** Pulse centrifuge the BCS plate to 250 xg.
- ☐ **8.** Heat-seal the ASE plate. Pulse centrifuge to 250 xg.
- ☐ **9.** Vortex the plate at 1600 rpm for 1 minute.
- **10.** If you are using the heat block for hybridization:
  - ☐ **a.** Place the sealed ASE plate on the 70°C heat block and close the cover.
  - ☐ **b.** Immediately reset the temperature to 30°C.
  - ☐ **c.** Allow the ASE plate to cool to 30°C. Allow about 2 hours.

11. If you are using the hybridization oven for hybridization:

- ☐ **a.** Set the high-speed shaker in the oven to 1350 rpm, shaking for 10 seconds on, 50 seconds off, overnight.
- ☐ **b.** Place the ASE plate on the shaker and secure it with straps.
- ☐ **c.** Press **Pulse On**.
- ☐ **d.** Start Program 1. The temperature ramps slowly from 70°C to 30°C.
- ☐ **e.** Leave the plate in the hyb oven for at least 16 hours.

Good stopping point

Next step

Proceed to Add MEL.

Add MEL

Estimated processing time: 30 minutes

Incubation time: 15 minutes

Extend and ligate the bound primers from the ASO to the LSO to create a single strand of amplifiable DNA template.

New Materials	Quantity
MEL	1 tube per plate, -20°C
AM1	Bottle, 4°C
UB1	Bottle, 4°C

Preparation

- ☐ Preheat the heat block to 45°C. Allow an hour for the temperature to stabilize.
- ☐ Thaw the MEL reagent to 22°C.
- ☐ Pour 11 ml AM1 into a reagent reservoir, plus 10 ml for each additional plate.
- ☐ Pour 11 ml UB1 into another reagent reservoir, plus 10 ml for each additional plate.

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Steps

In this procedure, you will remove all the liquid from the wells several times, leaving only the beads. Work quickly so that the beads do not dry out.

- [ ] **1.** Centrifuge the ASE plate to 250 xg.
- [ ] **2.** Place the ASE plate on a magnetic plate for 2 minutes or until the beads are completely captured.
- [ ] **3.** Leaving the ASE plate on the magnetic plate, remove and discard all liquid (about 50 µl) from the wells. Only the beads should remain.
- [ ] **4.** Leaving the ASE plate on the magnetic plate, add 50 µl AM1 to each well.
- [ ] **5.** Seal the ASE plate with adhesive film.
- [ ] **6.** Vortex at 1600 rpm for 20 seconds or until all beads are resuspended.
- [ ] **7.** Place the ASE plate on a magnetic plate for 2 minutes, until the beads are completely captured.
- [ ] **8.** Remove and discard the AM1 (about 50 µl) from the wells. Only the beads should remain.
- [ ] **9.** Repeat the process of adding, vortexing, magnetizing, and removing AM1 one time.

Repeat once

- [ ] **10.** Remove the ASE plate from the magnetic plate. Add 50 µl UB1 to each well.
- [ ] **11.** Place the ASE plate on a magnetic plate for 2 minutes, until the beads are completely captured.
- [ ] **12.** Remove and discard the UB1 (about 50 µl) from the wells. Only the beads should remain.
- [ ] **13.** Repeat the process of adding, magnetizing, and removing UB1 one time.

Repeat once

- [ ] **14.** Add 37 µl MEL to each well of the ASE plate.
- [ ] **15.** Seal the plate with adhesive film.
- [ ] **16.** Vortex at 1725 rpm for 1 minute or until the beads are resuspended.
- [ ] **17.** Incubate the ASE plate on the 45°C heat block for 15 minutes.

Next step

Do one of the following:

- Proceed to Make PCR immediately, leaving the ASE plate at 22°C until needed.
- Store the ASE plate at 4°C for up to one hour.

Make PCR

Estimated processing time: 15 minutes

Create a plate that contains a mixture for PCR.

New Materials	Quantity
96-well 0.2 ml microtiter plate	1 plate for each ASE plate
MMP	1 tube per plate, -20°C
Polymerase Enzyme	1 tube (64 µl), -20°C
UDG (optional)	1 tube (50 µl), -20°C

Preparation

- ☐ Thaw the MMP reagent to 22°C.
- ☐ Apply a PCR barcode to a new TCY plate. Create one PCR plate for each ASE plate.

Steps

- ☐ **1.** Add 64 µl enzyme to each MMP tube. Invert several times to mix.
- ☐ **2.** (Optional) Add 50 µl UDG to the MMP tube.
- ☐ **3.** Invert the MMP tube several times. Pour into a reagent reservoir.
- ☐ **4.** Add 30 µl of the enhanced MMP solution to each well of the PCR plate.
- ☐ **5.** Seal the plate with adhesive film.
- ☐ **6.** Pulse centrifuge to 250 xg.

Next step

Proceed to Inoc PCR.

Inoc PCR

Estimated processing time: 30 minutes

Wash the beads in the ASE plate. Separate the amplifiable template from the genomic DNA on the beads and transfer it to the PCR plate.

New Materials	Quantity
IP1	1 tube per plate, -20°C
UB1	Bottle (6 ml), 4°C

Preparation

- ☐ Remove the ASE plate from the 45°C heat block. Reset the heat block to 95°C.
- ☐ Pour 6 ml UB1 into a reagent reservoir.
- ☐ Pour the IP1 tube into another reagent reservoir.

Steps

- ☐ **1.** Place the ASE plate on a magnetic plate for 2 minutes or until all the beads are captured.
- ☐ **2.** Leaving the ASE plate on the magnetic plate, remove and discard all liquid (about 50 µl) from the wells.
- ☐ **3.** Add 50 µl UB1 to each well. Leave the ASE plate on the magnetic plate for 2 minutes.
- ☐ **4.** Remove and discard the UB1 (about 50 µl) from the wells.
- ☐ **5.** Add 35 µl IP1 to each well of the ASE plate.
- ☐ **6.** Seal the plate with adhesive film.
- ☐ **7.** Vortex at 1800 rpm for 1 minute or until all the beads are resuspended.
- ☐ **8.** Place the ASE plate on the 95°C heat block for 1 minute.
- ☐ **9.** Place the ASE plate on a magnetic plate for 2 minutes or until all the beads have settled.
- ☐ **10.** Transfer 30 µl of the supernatant from each well of the ASE plate to the corresponding well of the PCR plate.
- ☐ **11.** Seal the PCR plate with Microseal "A" PCR plate-sealing film.
- ☐ **12.** Place the PCR plate and the experienced user cards in a transfer box for the post-PCR area.

Next step

Proceed to Cycle PCR.

This is the end of Pre-PCR. Do not return the plates or experienced user cards to the Pre-PCR area at any time.

Cycle PCR

Estimated processing time: 2.75 hours

Amplify the template DNA.

Steps

☐ **1.** Place each sealed PCR plate into a thermal cycler and run the following program.

	Temperature	Time
	37°C	10 minutes
	95°C	3 minutes
Repeat 33 times	95°C	35 seconds
	56°C	35 seconds
	72°C	2 minutes
	72°C	10 minutes
	4°C	5 minutes

☐ **2.** Remove each PCR plate from the thermal cycler as soon as the program finishes running.

Do not splash!

☐ **3.** Remove the Microseal "A" film and replace with Microseal "F" film.

Good stopping point

Next step

Do one of the following:

- Proceed to Bind PCR. Store the PCR plate at 22°C, protected from light.
- Store the PCR plate at -20°C overnight.

Bind PCR

Estimated processing time: 30 minutes
Incubation time: 1 hour

Bind the biotinylated, fluorescent amplicons to paramagnetic beads.

New Materials	Quantity
Filter plate with lid	1 plate and lid for each PCR plate
MPB	1 tube per plate, 4°C

Preparation

- ☐ Apply a Filter Plate: PCR barcode label to the microtiter plate.
- ☐ In the space provided on the filter plate label, write in the same barcode number that appears on the PCR label.

Steps

1. ☐ Vortex MPB until the beads are completely resuspended.
2. ☐ Pulse centrifuge the PCR plate to 250 xg.
3. ☐ Add 20 µl resuspended MPB into each well of the PCR plate.
4. ☐ Pipet the solution in the wells of the PCR plate up and down several times.

   Tip: Set the pipette to 85 µl to allow space for bubbles.
5. ☐ Transfer the contents of each well of the PCR plate to the corresponding well in the filter plate. There should be about 70 µl fluid in each well.
6. ☐ Incubate the covered filter plate at 22°C for 60 minutes in a light-protected drawer.

Next step

Proceed to Make Hyb.

Make Hyb

Estimated processing time: 45 minutes

Wash away unused dyes, separate the biotin-labelled amplicons from the beads, and discard the beads. Create a 384-well hybridization plate that contains the sample and a humidifying solution in separate wells.

New Materials	Quantity
96-well V-bottom plate	2 plates for each PCR plate (one is for waste)
Cliniplate 384-well microplate	1 plate for each PCR plate
Filter plate adaptor	1 adaptor for each PCR plate
MH1	1 tube per plate, 22°C
UB2	Bottle (10 ml), 22°C
0.1N NaOH	Bottle (5ml), 4°C

Preparation

- ☐ Pour 10 ml UB2 into a reagent reservoir.
- ☐ Pour 5 ml 0.1N NaOH into a reagent reservoir.
- ☐ Pour the MH1 tube into a reagent reservoir.
- ☐ Apply an INT barcode label to one of the 96-well V-bottom plates.
- ☐ Apply a HYB barcode label to each new 384-well microplate.
- ☐ If you plan to proceed immediately to Hyb SAM, then start preheating the hybridization oven to 60°C.

Steps

- ☐ **1.** Place the covered filter plate onto the filter plate adaptor, and place the adaptor onto one of the 96-well V-bottom plates.
- ☐ **2.** Centrifuge the plate assembly to 1000 xg for 5 minutes at 25°C.
- ☐ **3.** Add 50 µl UB2 to each well of the filter plate.
- ☐ **4.** Replace the lid. Centrifuge to 1000 xg for 5 minutes at 25°C.
- ☐ **5.** Add 30 µl MH1 to each well of the INT plate.
- ☐ **6.** Replace the waste plate with the INT plate. Discard the waste plate.
- ☐ **7.** Add 30 µl 0.1N NaOH to each well of the filter plate.
- ☐ **8.** Replace the lid on the filter plate. Immediately centrifuge to 1000 xg for 5 minutes at 25°C. Discard the filter plate.

- [ ] **9.** Gently move the INT plate from side to side without splashing.
- [ ] **10.** Using the Humidity Control Template underneath the HYB plate as a guide, add 30 µl UB2 to the wells indicated by the orange dots.
- [ ] **11.** Pipet the sample in the wells of the INT plate up and down several times to completely neutralize the pH.
- [ ] **12.** Using the Sample Wells Template underneath the HYB plate as a guide, transfer 50 µl neutralized hyb solution from each well of the INT plate into the wells indicated by the blue dots.
- [ ] **13.** Seal the HYB plate with adhesive film. Centrifuge to 3000 xg for 4 minutes at 25°C to remove any bubbles.

Good stopping point

Next step

Do one of the following:

- Proceed to Hyb SAM.
- Store the HYB plate at -20°C overnight.

Hyb SAM

Estimated processing time: 30 minutes
Incubation time: 14.5 hours

Prepare the Sentrix® Array Matrix (SAM). Hybridize the sample to the SAM.

New Materials	Quantity
SAM (Sentrix Array Matrix)	1 for each HYB plate
SAM Hyb Cartridge	1 for each SAM
OmniTray	2 trays for each SAM
UB2	Bottle (70 ml), 22°C
0.1N NaOH	Bottle (60 ml), 4°C

Preparation

- [ ] Preheat the hybridization oven to 60°C (Program 2). Allow 45 minutes.
- [ ] If you froze the HYB plate after Make Hyb, thaw it completely at 22°C in a light-protected drawer. Centrifuge the plate to 3000 xg for 4 minutes.

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- ☐ Pour 70 ml UB2 into the first OmniTray. Label the tray "UB2."
- ☐ Pour 60 ml 0.1N NaOH into the second OmniTray. Label the tray "NaOH."
- ☐ Unpackage the SAM. Do not touch the fiber bundles. Put the decode CD in a safe place.

Steps

- ☐ **1.** Place the SAM into the UB2 tray, with the fiber bundles pointing down and the barcode label facing up.
- ☐ **2.** Gently move the SAM up and down for 10 seconds to remove bubbles from the ends of the fiber bundles. Set the timer for 3 minutes.
- ☐ **3.** After 3 minutes, move the SAM into the NaOH tray. Set the timer for 30 seconds.
- ☐ **4.** After 30 seconds, move the SAM back into the UB2 tray. Set the timer for 30 seconds.
- ☐ **5.** Place the HYB plate into the SAM Hyb Cartridge.
- ☐ **6.** Place the SAM in the Hyb Cartridge so that the fiber bundles extend down into the sample.
- ☐ **7.** Close the lid of the Hyb Cartridge.
- ☐ **8.** Incubate the HYB/SAM pair in the 60°C oven for 30 minutes.
- ☐ **9.** Reset the oven to 45°C.
- ☐ **10.** Incubate the HYB/SAM pair in the 45°C oven for at least 14 hours.

Next step

Proceed to Image SAM.

Image SAM

Estimated processing time: 2 hours per SAM
Dry time: 20 minutes

Image the hybridized SAM using the BeadArray™ Reader, which records the color of the fluorophores associated with each bead in the fiberoptic bundles.

New Materials	Quantity
OmniTray	4 trays for each SAM
IS1	Bottle, 22°C
UB2	Bottle (140 ml), 22°C
95% EtOH	*As needed*
Sec-butanol	*As needed*

Preparation

- ☐ Turn on the BeadArray Reader and let it initialize for at least one or two hours.

Steps

- ☐ **1.** Make a 50:50 mixture of sec-butanol and 95% EtOH.
- ☐ **2.** Add 940 ml of the sec-butanol/EtOH mixture to 60 ml of IS1 reagent, or 94 ml to 6 ml of IS1. Invert several times to mix.

  Do not reuse either the solution or the bottle. IS1 contains a photosensitive coating that protects the SAM from light, and the coating quickly degrades once exposed.
- ☐ **3.** Pour 70 ml UB2 into two OmniTrays. Label the trays "UB2."
- ☐ **4.** Pour 70 ml IS1 into the third OmniTray. Label the tray "IS1."
- ☐ **5.** Lift the SAM out of the HYB plate. Check for anomalies such as dry fiber bundles or crystals on the base of the array.
- ☐ **6.** Place the SAM into the first UB2 tray. Gently agitate 10 times and then set the timer for 1 minute.
- ☐ **7.** Place the SAM into the second UB2 tray and set the timer for 1 minute. Gently agitate 10 times and then let the array sit.
- ☐ **8.** Place the SAM into the IS1 tray for 5 minutes. Lift the SAM up and down a few times to ensure complete buffer exchange.
- ☐ **9.** Place the SAM upside-down on an empty OmniTray so that the fibers point upwards. Air-dry for at least 20 minutes.
- ☐ **10.** Seal the HYB plate with adhesive film and store it at -20°C.
- ☐ **11.** Clean the non-bead ends of the SAM fiber bundles with canned air.

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- [ ] **12.** At the BeadArray Reader PC, copy the decode data from the CD that came with the SAM into the decode folder identified in the BeadArray settings.
- [ ] **13.** Place the SAM in the scanner tray and begin the scan.

  Store the other SAMs in a dehumidifying chamber or a light-protected drawer. Image the SAMs within 24 hours.